

The above probes are incubated in approximately 5 molar excess of the target polynucleotide ends as follows: the probes are incubated for 60 minutes at 16° C. with 200 units of T4 DNA ligase and the anchored target polynucleotide in T4 DNA ligase buffer, after washing, the target polynucleotide is then incubated with 100 units T4 polynucleotide kinase in the manufacturer's recommended buffer for 30 minutes at 37° C., washed, and again incubated for 30 minutes at 16° C. with 200 units of T4 DNA ligase and the anchored target polynucleotide in T4 DNA ligase buffer. Washing is accomplished by successively flowing volumes of wash buffer over the slide, e.g. TE, disclosed in Sambrook et al (cited above). After the cycle of ligation-phosphorylation-ligation and a final washing, the attached microparticles are scanned for the presence of fluorescent label, the positions and characteristics of which are recorded by the scanning system. The labeled target polynucleotide, i.e. the ligated complex, is then incubated with 10 units of Fok I in the manufacturer's recommended buffer for 30 minutes at 37° C., followed by washing in TE. As a result the target polynucleotide is shortened by one nucleotide on each strand and is ready for the next cycle of ligation and cleavage. The process is continued until twenty nucleotides are identified.

APPENDIX I

Exemplary computer program for generating
minimally cross hybridizing sets

```

Program minxh
c
c
integer*2 sub1(6),mset1(1000,6),mset2(1000,6)
dimension nbase(6)

c
write(*,*)'ENTER SUBUNIT LENGTH'
read(*,100)nsub
format(i1)
open(1,file='sub4.dat',form='formatted',status='new')

c
nset=0
do 7000 m1=1,3
    do 7000 m2=1,3
        do 7000 m3=1,3
            do 7000 m4=1,3
                sub1(1)=m1
                sub1(2)=m2
                sub1(3)=m3
                sub1(4)=m4
            c
            c
            ndiff=3

            Generate set of subunits differing from
            sub1 by at least ndiff nucleotides.
            Save in mset1.

            jj=1
            do 900 J=1,nsub
                mset1(1,j)=sub1(j)

900      do 1000 k1=1,3
                    do 1000 k2=1,3
                        do 1000 k3=1,3
                            do 1000 k4=1,3
```

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```

5  c
  c
      nbase(1)=k1
      nbase(2)=k2
      nbase(3)=k3
10  nbase(4)=k4
  c
      n=0
      do 1200 j=1,nsub
          if(sub1(j).eq.1.and.nbase(j).ne.1.or.
1      sub1(j).eq.2.and.nbase(j).ne.2.or.
15  3      sub1(j).eq.3.and.nbase(j).ne.3) then
              n=n+1
              endif
          continue
      1200
  c
  c
      if(n.ge.ndiff) then
10  c
      c
      c          If number of mismatches
      c          is greater than or equal
      c          to ndiff then record
      c          subunit in matrix mset
15  c
          jj=jj+1
          do 1100 i=1,nsub
              mset1(jj,i)=nbase(i)
          1100
          endif
      c
      c
      c          continue
      c
      c
          do 1325 j2=1,nsub
              mset2(1,j2)=mset1(1,j2)
5      1325
              mset2(2,j2)=mset1(2,j2)
      c
      c
      c          Compare subunit 2 from
      c          mset1 with each successive
      c          subunit in mset1, i.e. 3,
      c          4,5, ... etc. Save those
      c          with mismatches .ge. ndiff
      c          in matrix mset2 starting at
      c          position 2.
      c          Next transfer contents
      c          of mset2 into mset1 and
      c          start
5      c          comparisons again this time
      c          starting with subunit 3.
      c          Continue until all subunits
      c          undergo the comparisons.
      c
      c
      c          npass=0
      c
      c
      c          continue
      c          kk=npass+2
      c          npass=npass+1
5      c
      c
          do 1500 m=npass+2,jj
              n=0
              do 1600 j=1,nsub
                  if(mset1(npass+1,j).eq.1.and.mset1(m,j).ne.1.or.
0      2      mset1(npass+1,j).eq.2.and.mset1(m,j).ne.2.or.
0      2      mset1(npass+1,j).eq.3.and.mset1(m,j).ne.3) then
                      n=n+1
                      endif
                  continue
              1600
                  if(n.ge.ndiff) then
                      kk=kk+1
                      do 1625 i=1,nsub
                          1625
                              mset2(kk,i)=mset1(m,i)

```

-continued

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1500 endif
 continue
c
c
c
c kk is the number of subunits
 stored in mset2
c
c Transfer contents of mset2
 into mset1 for next pass.
c
c
c do 2000 k=1,kk
 do 2000 m=1,nsub
2000 mset1(k, m)=mset2 (k, m)
 if(kk.lt.ij) then
 ij=kk
 goto 1700
 endif
c
c
c nset=nset+1
 write (1,7009)
7009 format(/)
 do 7008 k=1,kk
7008 write(1,7010)(mset1(k,m),m=1,nsub)
7010 format(4i1)
 write(*,*)
 write(*,120) kk,nset
120 format(1x,'Subunits in set=',i5,2x,'Set No=',i5)

SUB
cle

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APPENDIX I

Exemplary computer program for generating
minimally cross hybridizing sets

7000 continue

```
close(1)
```

10

C

end

15

20

25

SUB.
C4